

Claims

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1. Process for the production of S-layer proteins
w h e r e i n
- (a) a gram-negative prokaryotic host cell is
provided which is transformed with a nucleic
acid coding for an S-layer protein which is
selected from
 - (i) a nucleic acid which comprises the
nucleotide sequence from position 1 to 3684
shown in SEQ ID NO.1 optionally without the
signal peptide-coding section,
 - (ii) a nucleic acid which comprises a nucleotide
sequence corresponding to the nucleic acid
from (i) within the scope of the degeneracy
of the genetic code and
 - (iii) a nucleic acid which comprises a nucleotide
sequence which hybridizes with the nucleic
acids from (i) or/and (ii) under stringent
conditions;
 - (b) the host cell is cultured under conditions which
lead to an expression of the nucleic acid and to
production of the polypeptide coded by it and
 - (c) the resulting polypeptide is isolated from the
host cell.
2. Process as claimed in claim 1,
w h e r e i n
an E. coli host cell is used.

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3. Process as claimed in claim 1 or ~~2~~,
w h e r e i n
the polypeptide is isolated from the interior of
the host cell in the form of an assembled S-layer
structure....
4. Process as claimed in ~~one of the claims 1 to 3~~,
w h e r e i n
the nucleic acid coding for the S-layer protein
contains one or several insertions which code for
peptide or polypeptide sequences.
5. Process as claimed in claim 4,
w h e r e i n
the insertions are selected from nucleotide
sequences which code for cysteine residues, regions
with several charged amino acids or Tyr residues,
DNA-binding epitopes, metal-binding epitopes,
immunogenic epitopes, allergenic epitopes,
antigenic epitopes, streptavidin, enzymes,
cytokines or antibody-binding proteins.
6. Process as claimed in claim 5,
w h e r e i n
the insertions code for streptavidin.
7. Process as claimed in claim 5,
w h e r e i n
the insertions code for immunogenic epitopes from
herpes viruses, in particular herpes virus 6 or
FMDV.

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8. Process as claimed in claim 5,
w h e r e i n
the insertions code for enzymes such as
polyhydroxybutyric acid synthase or bacterial
luciferase.
9. Process as claimed in claim 5,
w h e r e i n
the insertions code for cytokines such as
interleukins, interferons or tumour necrosis
factors.
10. Process as claimed in claim 5,
w h e r e i n
the insertions code for antibody-binding proteins
such as protein A or protein G.
11. Process as claimed in claim 5,
w h e r e i n
the insertions code for antigenic epitopes which
bind cytokines or endotoxins.
12. Process as claimed in claim 5,
w h e r e i n
the insertions code for metal-binding epitopes.
13. Process as claimed in ^{C/A/m /} ~~one of the claims 1 to 12,~~
w h e r e i n
a nucleic acid coding for a gram-positive signal
peptide is arranged in operative linkage at the 5'
side of the nucleic acid coding for the S-layer
protein.

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14. Process as claimed in claim 13,
w h e r e i n
the nucleic acid coding for the signal peptide
comprises
- (a) the signal peptide-coding section of the
nucleotide sequence shown in SEQ ID NO.1,
 - (b) a nucleotide sequence corresponding to the
sequence from (a) within the degeneracy of the
genetic code or/and
 - (c) a nucleotide sequence that is at least 80 %
homologous to the sequences from (a) or/and
(b).
15. Nucleic acid that codes for a recombinant S-layer
protein and is selected from
- (i) a nucleic acid which comprises the nucleotide
sequence from position 1 to 3684 shown in
SEQ ID NO.1 optionally without the signal
peptide-coding section,
 - (ii) a nucleic acid which comprises a nucleotide
sequence corresponding to the nucleic acid
from (i) within the scope of the degeneracy
of the genetic code and
 - (iii) a nucleic acid which comprises a nucleotide
sequence which hybridizes with one of the
nucleic acids from (i) or/and (ii) under
stringent conditions,
- wherein the nucleic acid contains at least one
peptide or polypeptide-coding insertion within the
region coding for the S-layer protein.
16. Nucleic acid as claimed in claim 15,
w h e r e i n
the insertion site is located at position 582, 878,

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917, 2504 or/and 2649 of the nucleotide sequence shown in SEQ ID NO.1.

17. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as claimed in claim 15 ~~or 16.~~

18. Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in claim 15 ~~or 16~~ or with a vector as claimed in claim 17.

19. Cell as claimed in claim 18,
w h e r e i n
it is a gram-negative prokaryotic cell and in particular an E. coli cell.

20. Cell as claimed in claim 18 ~~or 19,~~
w h e r e i n
it contains a recombinant S-layer structure.

21. Recombinant S-layer protein,
w h e r e i n
it is coded by a nucleic acid as claimed in claim 15 ~~or 16.~~

22. Recombinant S-layer structure,
w h e r e i n
it contains at least one protein as claimed in claim 21 as a subunit.

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23. S-layer structure as claimed in claim 22,
w h e r e i n
it additionally contains at least one unmodified S-
layer protein as a subunit.
24. S-layer structure as claimed in claim 22 ~~or 23,~~
w h e r e i n
it comprises several layers which are linked
covalently or by affinity binding.
25. Use of an S-layer protein as claimed in claim 21 or
an S-layer structure as claimed in one of the claims
22 to 24 as a vaccine or adjuvant.
26. Use as claimed in claim 25,
w h e r e i n
the vaccine or adjuvant additionally comprise a
bacterial ghost which optionally contains further
immunogenic epitopes in its membrane.
27. Use of an S-layer protein as claimed in claim 21 or
an S-layer structure as claimed in one of the claims
22 to 24 as an enzyme reactor.
28. Nucleic acid which codes for an S-layer protein and
is selected from
(i) a nucleic acid which comprises the nucleotide
sequence from position 1 to 2763 shown in SEQ
ID NO.5 optionally without the signal peptide-
coding section,
(ii) a nucleic acid which comprises a nucleotide
sequence corresponding to the nucleic acid from
(i) within the scope of the degeneracy of the
genetic code and

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(iii) a nucleic acid which comprises a nucleotide sequence that hybridizes with the nucleic acids from (i) or/and (ii) under stringent conditions.

29. Nucleic acid as claimed in claim 28,
w h e r e i n
it contains at least one peptide-coding or polypeptide-coding insertion within the region coding for the S-layer protein.
30. Vector,
w h e r e i n
it contains at least one copy of a nucleic acid as claimed in claim 28 ~~or 29~~.
31. Cell,
w h e r e i n
it is transformed with a nucleic acid as claimed in claim 28 or 29 ~~or~~ with a vector as claimed in claim 30.
32. Cell as claimed in claim 31,
w h e r e i n
it contains a recombinant S-layer structure.
33. S-layer protein,
w h e r e i n
it is coded by a nucleic acid as claimed in claim 29.

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34. Recombinant S-layer structure,
w h e r e i n
it contains at least one recombinant S-layer
protein as a subunit which is coded by a nucleic
acid as claimed in claim 29.
35. Use of an S-layer protein as claimed in claim 33 or
of an S-layer structure as claimed in claim 34 as a
vaccine or adjuvant.
36. Use of an S-layer protein as claimed in claim 33 or
an S-layer structure as claimed in claim 34 as an
enzyme reactor.
37. Process for the production of recombinant S-layer
proteins,
w h e r e i n
(a) a host cell is provided which contains a
nucleic acid coding for an S-layer protein
which contains a peptide-coding or polypeptide-
coding insertion within the region coding for
the S-layer protein,
(b) the host cell is cultured under conditions
which lead to an expression of the nucleic acid
and to production of the polypeptide coded by
it and
(c) the resulting polypeptide is isolated from the
host cell or from the culture medium.
38. Process as claimed in claim 37,
w h e r e i n
the nucleic acid coding for the recombinant S-layer
protein is selected from
(i) a nucleic acid which comprises the nucleotide

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sequence from position 1 to 3684 shown in SEQ ID NO.1 optionally without the signal peptide-coding section,

- (ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and
- (iii) a nucleic acid which comprises a nucleotide sequence which hybridizes with one of the nucleic acids from (i) or/and (ii) under stringent conditions

39. Process as claimed in claim 37,

w h e r e i n

the nucleic acid which codes for the recombinant S-layer protein is selected from

- (i) a nucleic acid which comprises the nucleotide sequence from position 1 to 2763 shown in SEQ ID NO.5 optionally without the signal peptide-coding section,
- (ii) a nucleic acid which comprises a nucleotide sequence corresponding to the nucleic acid from (i) within the scope of the degeneracy of the genetic code and
- (iii) a nucleic acid which comprises a nucleotide sequence that hybridizes with the nucleic acids from (i) or/and (ii) under stringent conditions.

40. Process as claimed in one of the ^{claim 37}~~claims 37-39~~,

w h e r e i n

a further S-layer gene is expressed in the host cell which codes for an unmodified S-layer protein.

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